Microbial Community and Environmental Factors Affecting Copper Complexation in a Navy Harbor

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LONG-TERM GOAL

Our long-term goal is to understand the interactions between microorganisms and copper in estuaries that are heavily utilized by naval operations. We are particularly interested in the production of high-affinity, copper-complexing ligands by microbial populations in response to elevated copper concentrations.

OBJECTIVES

Strong, dissolved, copper-complexing ligands are known to control copper speciation and bioavailability in most marine waters. We are testing the hypothesis that metal-responsive production of such ligands occurs in the Elizabeth River estuary, and that picoplankton and bacterioplankton produce the ligands. Recent studies utilizing cultures of marine picoplankton (*Synechococcus*) and bacterioplankton (*Vibrio*) have demonstrated that, when the cultures are exposed to elevated copper concentrations, these microbes produce copper-complexing ligands having copper-binding strengths similar to those found in marine waters. The primary objective of our study is to extend these observations to field conditions and natural assemblages of estuarine microorganisms in a naval harbor (the Elizabeth River, home of the US Atlantic Fleet). An additional objective is isolation and further characterization of the strong, copper-complexing ligands produced by microbes.

APPROACH

We have carried out *in situ* incubations of water samples collected adjacent to Pier 12 at the Norfolk Naval Base (Sewell's Point). Incubations were performed at three times: May and November 2000 and May/June 2001 under comparable conditions. Navy clearance was required and obtained for placing our incubation apparatus (moored array holding up to 20, two-liter incubation bottles) at the naval base site. Trace metal clean sampling procedures were utilized to collect water from each site. Treatments included copper additions at concentrations of 100 and 200 nM, filtration (0.2 μm and 3 μm), sodium

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14. ABSTRACT
Our long-term goal is to understand the interactions between microorganisms and copper in estuaries that are heavily utilized by naval operations. We are particularly interested in the production of high-affinity, copper-complexing ligands by microbial populations in response to elevated copper concentrations.

15. SUBJECT TERMS

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azide addition and light/dark incubation. The effect of the treatments on the microbial community, copper speciation, and copper-complexing ligand concentration was determined.

WORK COMPLETED

We completed analyses of samples from the May and November 2000 incubations, and we performed an incubation during May/June 2001. Analyses of samples from the last incubation are nearing completion. The data show that microbial communities *in situ* can actively respond to elevated copper concentrations by production of copper-complexing ligands. The results indicate that heterotrophic bacteria are the primary contributors to the ligand pool in this environment.

We made progress on development of chromatographic procedures for isolation and characterization of copper-complexing ligands from waters collected at the Norfolk Naval Base study site.

RESULTS

We utilized a moored array for *in situ* incubations of intact microbial communities exposed to elevated copper levels. The moored array holds 20, two-liter polycarbonate incubation bottles at a constant depth of one meter below the surface. It is buoyed by floats at each corner and moored to the bottom by a line attached to a 15 inch mooring buoy at the top and a 150 lb. weight at the bottom. The array moves freely up or down the mooring line with the tide maintaining the bottles at a constant depth.

During the May and November 2000 incubations, copper-complexing ligand concentration increased in response to copper addition in a dose-dependent manner in bottles that contained the intact microbial community. Ligand concentrations did not increase in bottles with the metabolic inhibitor sodium azide added or bottles containing 0.2µm filtered water (Figure 1). Taken together, these results indicate that the intact microbial community was responsible for the increase in copper ligand concentrations. No notable difference in rate of ligand production was observed between seasons.

Removal of the > 3 µm component of the microbial community by pre-filtration prior to copper addition and incubation resulted in an <u>increase</u> in ligand accumulation at all copper concentrations (ambient, 100 and 200 nM) in the November 2000 incubation. Increased bacterial numbers were also observed in incubated samples that had been pre-filtered (3 µm). Removal of grazers is a likely explanation for increased bacterial abundance. These results indicate that *in situ* production of strong copper-complexing ligands in response to copper is primarily due to cells less than 3 µm in size. Such cells are primarily heterotrophic and phototrophic bacteria. Thus our results indicate that larger (> 3µm) phytoplankton such as diatoms, dinoflagellates and filamentous cyanobacteria do not actively contribute to copper-complexing ligand production in the Elizabeth River.

Similar rates of ligand production were observed in May and November 2000. The numbers of small, unicellular cyanobacteria (such as *Synechococcus*) differed by an order of magnitude (~1×10^8 /L in May and ~1×10^7 /L in November) while heterotrophic bacterial numbers were similar on both dates. This observation suggests that the heterotrophic bacteria are the principal ligand producers in the < 3 µm microbial community in this environment.

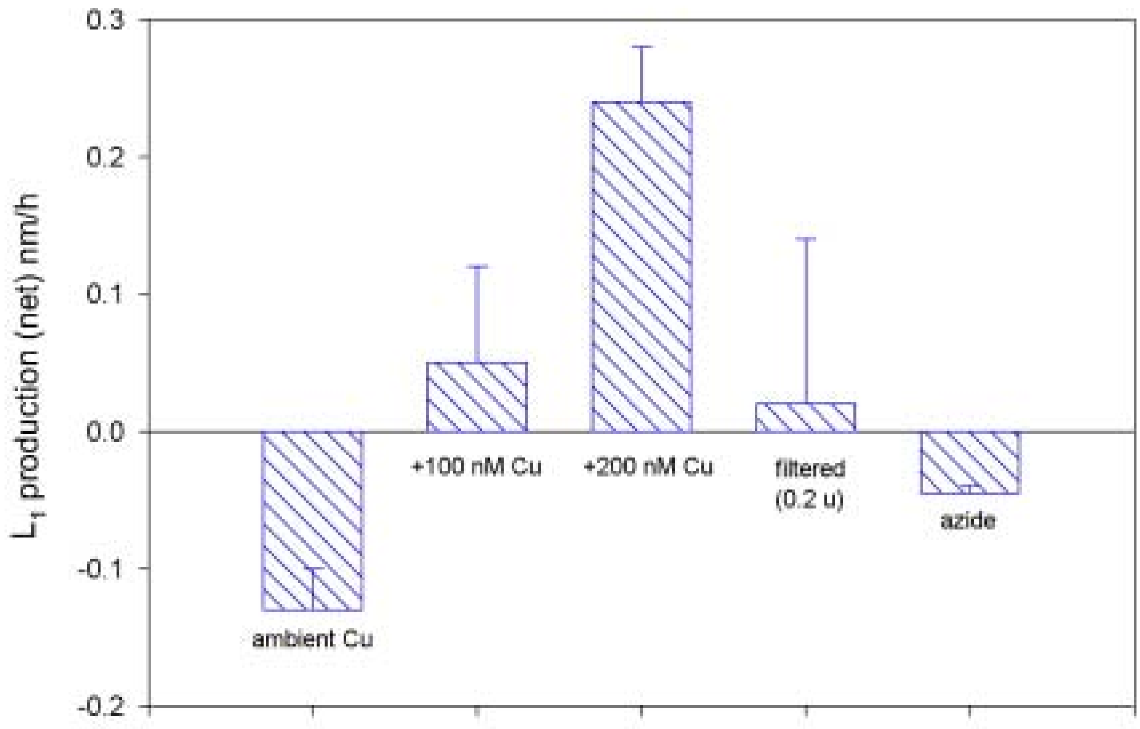

Figure 1. Concentration of strong, copper-complexing (L_1-class) ligands after a two- week in situ incubation of water samples subjected to various treatments. Biological activity was eliminated by addition of the metabolic poison sodium azide or by filtration. Changes in the rate of copper ligand production were positively correlated with the concentration of copper added and only observed after incubation when the microbial community was intact and active. Results from May 2000 and November 2000 incubations are averaged. Bars for the 0.2 μm filtered and azide treatments are averaged results for the three copper concentrations (ambient, 100 and 200 nM).

Analyses of data from the May/June 2001 incubation are nearing completion. Preliminary data from this incubation show that the rate of ligand production in bottles amended with 200 nM copper and incubated in the light and in the dark is the same. This supports our contention that heterotrophic bacteria are the most important producers of strong, copper-complexing ligands in this environment.

We have made excellent progress developing methods for isolation and characterization of copper-complexing ligands from estuarine water during the past year. Immobilized metal ion affinity chromatography (IMAC) was utilized to concentrate ligands from water collected from the Norfolk Naval Base study site. IMAC eluents demonstrated to contain L_1- class ligands were fractionated utilizing size exclusion HPLC-ICPMS (Figure 2). This separation strategy allowed detection of chromatographic peaks containing copper-complexing ligands. Analyses of fractions corresponding to ligand-containing peaks are in progress.

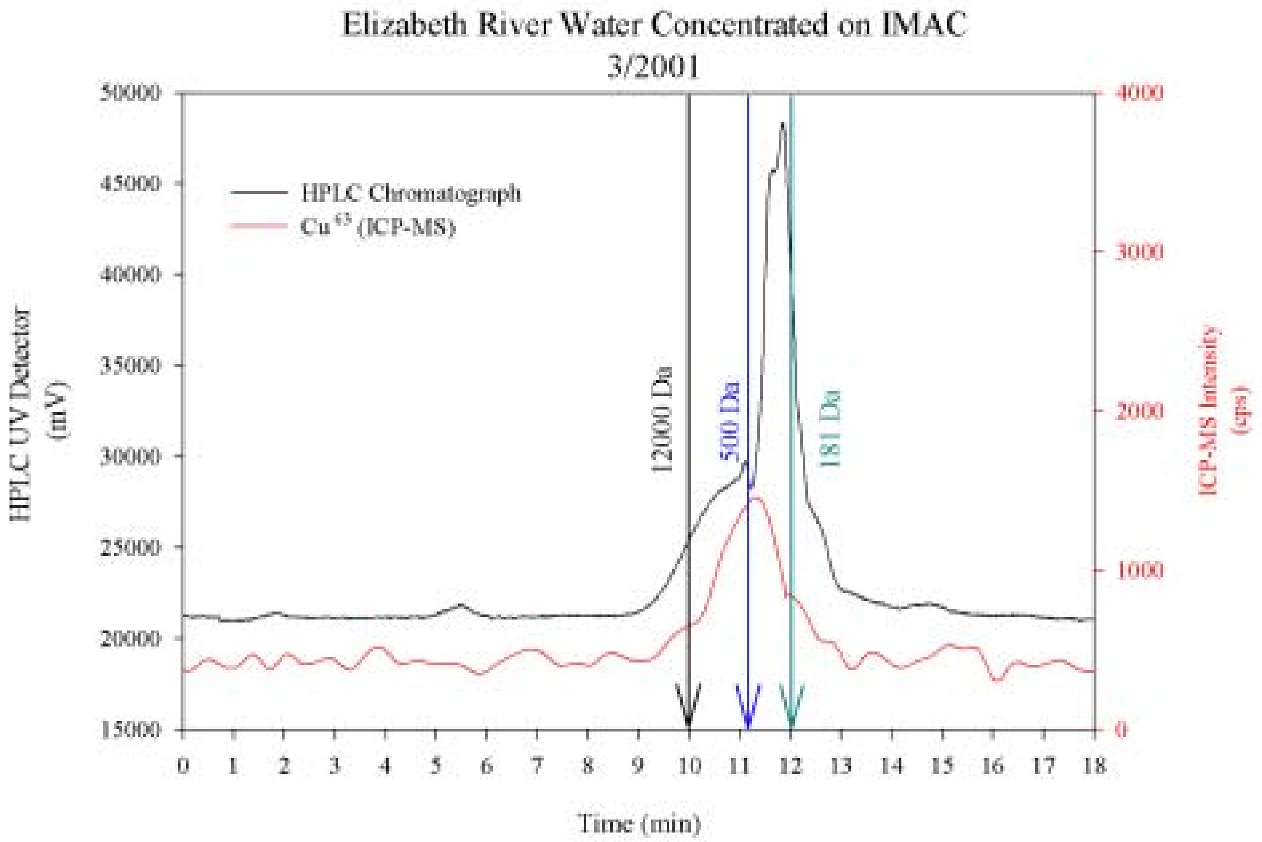

Figure 2. SEC-HPLC-ICPMS analysis of copper-complexing ligands pre-concentrated from Elizabeth River water by IMAC. The upper line is u.v. absorbance (220 nm) and the lower line is the relative copper concentration measured by ICP-MS. Copper-complexing ligands and copper co-elute in a fraction corresponding to a molecular mass of approximately 500 Da.

IMPACT/APPLICATION

This work has clarified the influence of microbial activity on copper complexation, speciation and bioavailability, and shown that microbial populations actively produce copper-complexing ligands in response to increased copper concentrations *in situ*. Our observation that heterotrophic bacteria are the primary active producers of strong, copper-complexing ligands in this environment has important implications for models regarding sources and dynamics of the ligands. These results will impact our thinking about the fate and effects of copper in estuarine ecosystems and naval harbors. A better understanding of how estuarine systems can respond to copper influx will allow for a more rational basis for setting concentration limits on effluents while protecting the health of the estuary.

RELATED PROJECTS

Concurrent, ONR-funded projects collectively entitled "Interactions among chemical speciation, algal accumulation, and sediment-water cycling of toxic metals in a major US Naval harbor (Elizabeth River, VA)", P.I. J.R. Donat, and Co-P.I. D.J. Burdige (Old Dominion University), and P.I. W.G. Sunda and Co-P.I. S.A. Huntsman (NMFS/NOAA, Beaufort NC Lab), are examining factors controlling algal metal uptake and accumulation, and the importance of sediment pore waters as a

source of metals and metal chelators to the overlying water. Since these projects are closely related, are examining the same site, and utilize some of the same methodologies as our project, all PIs are coordinating their efforts and sampling times to maximize the amount of information obtained. During July 1999 and May 2000 we coordinated sampling cruises on the Elizabeth River with deployment of our *in situ* incubation arrays. The selected sites for our incubation arrays were within the cruise track. In addition, during the May 2000 Elizabeth River sampling cruise, these P.I.'s were joined by researchers from ONR HP P.I. K. Buessler's group (WHOI), who are quantifying submarine groundwater discharge and contaminant fluxes in coastal harbors, and by Beth Ahner's research group (Cornell Univ), who are studying the relationships between phytochelatin concentrations and copper, cadmium, and zinc speciation. Data from these concurrent studies will be integrated to provide additional insight into the processes controlling microorganism trace-metal interactions in the Elizabeth River.

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